DIFFUSIVITY OF THREE-DIMENSIONAL, IONICALLY CROSSLINKED ALGINATE HYDROGELS

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Introduction

Tissue engineering offers great promise in creating biological body parts as alternatives for transplanting harvested tissues and organs[1]. In this approach, the scaffold, made of synthetic or natural polymers, provides the skeletal structure for cells that gradually develop into new tissue[2,3,4]. As the tissue forms, the scaffold degrades and eventually disappears completely. Because of their biocompatibility, abundance in source, and low prices, alginates have been widely used in the food industry as thickeners and emulsifying agents[5]. They have also been studied extensively for various biomedical applications. In particular, alginates have been processed into the form of calcium alginate gel beads for cell encapsulation. The procedure of crosslinking with calcium ions (e.g. $CaCl_2$ solution) is advantageous in that it is gentle for the cells, however the crosslinking is uncontrollably rapid and thus the process is limited to the fabrication of beads and results in gels with non-uniform structures. The development of homogeneous gels is crucial in tissue engineering to ensure structural integrity, uniform distribution of the cells, and also uniform porosity throughout the scaffold. Control over porosity and pore size distribution is necessary for selective permeability of these scaffolds. Nutrients need to be able to diffuse through the material to reach the cells. Similarly, waste products need to diffuse out. However, pore sizes that are too large will also provide entryway to immune cells that can harm allogenic cells and the developing tissue.

Previously, we presented ionically crosslinked alginate gels made with a slow-gelation system as excellent candidates for tissue engineering scaffolding[6,7]. We established the dependency of the gels' mechanical properties on the homogeneity of the gel, and with our methods demonstrated formation of homogeneous gels with uniform cell distribution throughout the scaffold. In this work we studied the influence of structural parameters on the diffusion of solutes of different sizes through the alginate gels.

Experimental

Gel Formation. Calcium alginate gels were made as previously described[6,7]. Briefly, sodium alginate was dissolved in deionized water for final gel concentrations of either 1.5 or 3.18% (w/v). The polymer solution was then mixed with calcium carbonate ($CaCO_3$) to form a suspension. A fresh aqueous solution of D-gluconic acid lactone (GDL) was then added to the suspension and mixed well to release the Ca^{2+} ions and initiate gelation. A $CaCO_3$ to GDL molar ratio of 0.5 was always maintained to achieve a neutral pH value. For all alginate gels, a basic calcium ion to carboxyl molar ratio of 0.18 was designated as 1X. The crosslinking density was adjusted with a multiplication factor to this molar ratio as a relative calcium ion content, such as 0.5X (molar ratio: 0.09). The calcium-alginate gels were made in Teflon vials and stored at room temperature in humidity for 48 h prior to experiments.

Diffusion. Diffusivity of vitamin B_{12} and FITC-dextran with molecular weights of 1355 and 9500, respectively, in alginate gels of high and low polymer concentrations were studied with diffusion experiments performed with Navicyte's Vertical Diffusion Chamber System. Gels were cut into samples about 2.9 mm in thickness. Each sample was placed between the donor and receptor chambers with a mesh on each side of the gel to hold it in place. The chambers were then connected to a gas manifold that delivered air flow of either 5% CO_2 and 95% O_2 for FITC-dextran, or N_2 for vitamin B_{12} to each chamber for constant mixing of the solutions. The experiment was carried out in an incubator set to 37°C and 5% CO_2. The concentration of the solution in the receptor chamber was determined by calculations based on measured absorbance values. Samples of 1.1 mL were taken directly from the chamber to a Shimadzu UV-265 UV-Vis spectrophotometer for measurement and then returned immediately to the receptor chamber. This was performed every 30 minutes for vitamin B_{12} samples, and every 60 minutes for FITC-dextran samples. Vitamin B_{12} samples were measured at 362 nm, while FITC-dextran samples were measured at 495 nm. The concentration of the donor solution was measured with 0.01 mL (for vitamin B_{12}) or 0.1 mL (for FITC-dextran) samples diluted with 1.0 mL water for measurement on the spectrophotometer. Experiments with vitamin B_{12} were run for 6 h, while those with FITC-dextran were run for 3 days. The lag time theory[8] was used to analyze the data collected and calculate the diffusion coefficient. When the average diffusion coefficients were compared, a two-tail Student *t* test (assuming unequal variances) was performed to determine the statistical significance ($p < 0.05$).

Results and Discussion

Diffusion of solutes of different molecular weights was studied with vitamin B_{12} and FITC-dextran molecules. The differences in diffusion coefficients obtained for vitamin B_{12} solutes through alginate gels of high and low polymer concentrations were not statistically significant (Figure 1). In contrast, the diffusion coefficient of the much larger FITC-dextran molecules decreased with the increase in polymer concentration, showing a statistically significant difference (Figure 2). At a constant crosslinking density, increasing the polymer concentration resulted in crowding and entanglement of polymer molecules. The increase in the number of polymer molecules and the degree of entanglement reduced the size of pores formed between the junctions and entangled molecules. This effect was seen in the difference between average diffusion coefficients measured for 1.5% *LH* 2X $CaCO_3$ and 3.18% *LH* 2X $CaCO_3$ gels (Figure 2).

The fast gelation system, such as that used in producing alginate gel beads, results in gels heterogeneous in structure. A heterogeneous gel would have an uneven distribution of porosity and pore sizes. Polymer molecules would be highly entangled in some regions and not so much in others. The areas of greater entanglement would also be highly crosslinked, effectively decreasing pore sizes further. The porosity in these areas would be extremely low, limiting diffusion of small molecules, such as nutrients. Other areas, however, would have pore sizes large enough to allow immune cells to easily enter the gel.

On the other hand, the slow gelation system produces homogeneous gels with uniform porosity and pore size distribution throughout the gels. Both polymer concentration and crosslinking density would be evenly distributed. Varying structural parameters such as alginate concentration would then control the molecular weight range of solutes that could then diffuse into the gel. This was seen in the increased difficulty of FITC-dextran diffusion through the gel with higher polymer concentration.

This and previous works show that ionically crosslinked calcium alginate gels formed with controllable mechanical properties, homogeneity, swelling behavior and permeability can be tailored specifically for tissue engineering or other biomedical applications.

References

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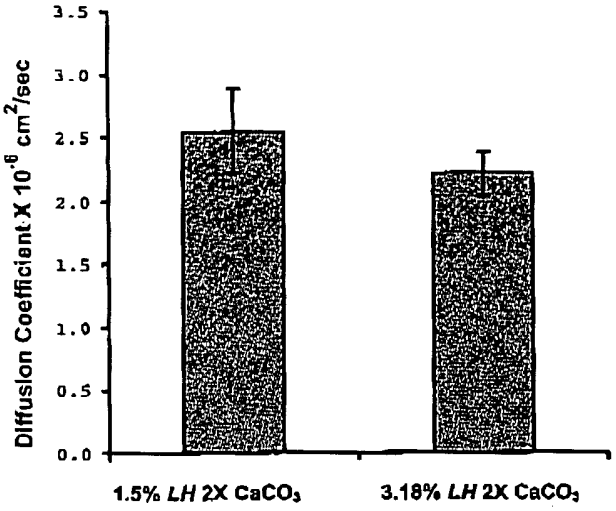

Figure 1. Diffusion coefficients of vitamin B_{12} through alginate gels of different polymer concentrations.

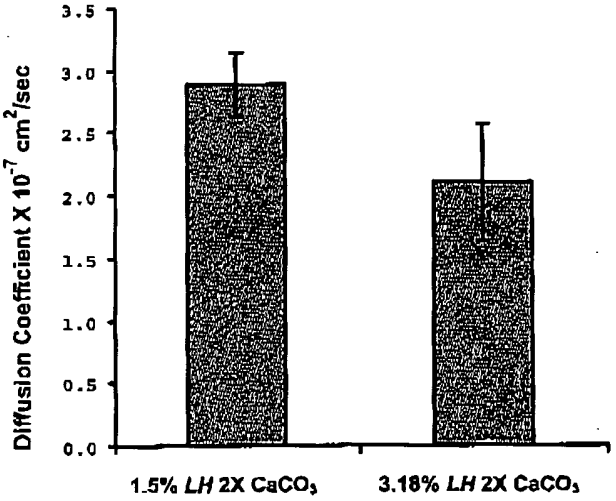

Figure 2. Diffusion coefficients of FITC-dextran through alginate gels of different polymer concentrations.